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Dated 19 January 2000

[redacted]s Fo: 1/77

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Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office
Cardiff Road
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1. Your reference
C1014/C

2. Patent application number
(The Patent Office will fill in this part)
9905954.5

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*
Cambridge Imaging Limited
St Johns Innovation Centre
Cowley Road
Cambridge CB4 4WS

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation
GB 2791860001

4. Title of the invention
Sample imaging

5. Name of your agent *(if you have one)*
Keith W Nash & Co

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*
90-92 Regent Street
Cambridge
CB2 1DP

Patents ADP number *(if you know it)*
1206001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*
a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))
Yes a)

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	0
Description	6
Claim(s)	0
Abstract	0
Drawing(s)	3

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature [signature] Date 16.3.99

Keith W Nash & Co, Agents

12. Name and daytime telephone number of person to contact in the United Kingdom

Mr Nash (01223) 355477

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.

b) Write your answers in capital letters using black ink or you may type them.

c) If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.

d) If you have answered 'Yes' Patents Form 7/77 will need to be filed.

e) Once you have filled in the form you must remember to sign and date it.

f) For details of the fee and ways to pay please contact the Patent Office.

C1014/C

Title: Sample imaging

This invention concerns optical systems for imaging multiwell sample plates and the like onto camera devices, for analysis and monitoring of light activity in the wells.

Biomedical samples, typically in multiwell sample plates, can be viewed and measured with a CCD camera using a suitable lens. The lens demagnification can be chosen to match the size of the whole sample plate (eg typically 110mm x 75mm), or a part of it, to the CCD. A CCD camera can be either a bare cooled CCD, or an image intensified CCD.

Typically a CCD camera sensor is 1" (25mm) square. A demagnification of ~110/25=4.4 is therefore necessary to view a whole sample plate.

In modern biomedical assay chemistries where luminescent or fluorescent light emission occurs at long wavelengths towards the red end of the spectrum (600-700mm) a bare cooled CCD has a great advantage. The CCD is cooled by Peltier or Cryogenic means to reduce the dark noise of the CCD sufficiently. Special electronics is needed to minimise read-out noise, but very low light levels can then be detected in the presence of low noise. The quantum efficiency of a CCD over most of the visible range is 35-40%. Using a thinned back-illuminated CCD, the efficiency can be as high as 80-90%.

The situation can be contrasted with image intensified CCD cameras, where photons are detected in the photocathode of the image intensifier. The quantum efficiency of typical low-noise photocathodes in the red is poor (<5%)). Where Gen1 image intensifiers are used, there is also usually shading, ie a fall-off of detection efficiency away from the centre of the field of view. Where Gen2 (microchannel plate) image

intensifiers are used, there is also a problem at medium and high light levels, where the tube lifetime becomes limited. Gen3 image intensifiers offer much improved quantum efficiency in the red, but these are to some extent in the development stage, at least where tubes of reasonable diameter (eg 40mm) are involved, and the noise level can be a problem.

With an image intensified CCD a single detected photon results in a burst of electrons in the CCD, spread over a number of pixels. Centroiding methods have been proposed to achieve sub-pixel spatial resolution (eg of the order of 10 microns) for locating the coordinates of a detected photon which is important in some imaging applications where many tiny light emitting sites are present in the sample, and the imaging process requires the different light emitting sites to be resolved the one from the other.

In general, centroiding methods cannot be used with a bare cooled CCD because a detected photon results in only a single electron in the silicon.

According to one aspect of the present invention, a sample plate is imaged onto a bare cooled CCD camera by at least one lens and a fibre-optic taper. A fibre optic taper possess some advantages and the use of a converging lens possesses other advantages. Optical arrangements embodying the invention therefore possess the advantages of both lens imaging and of contact imaging.

Preferably a shutter or iris is included in the light path between the sample and the CCD camera.

Where a single lens is employed, the shutter may be located between the lens and the CCD camera faceplate. Where two lenses are employed the shutter may be located between the two lenses.

In general a multiwell sample plate can be imaged and analysed in a single exposure (shot). However where a fibre optic taper is employed without a lens, two or more exposures (shots) may be required to form a complete the image and analyse a sample plate.

A system incorporating the invention is highly effective in light gathering. Efficiencies of the order of 3.5% can be envisaged.

A second lens may be incorporated to advantage. Typically an imaging lens is located close to the sample and a field lens is located close to the camera input faceplate.

Preferably the field lens bends the light rays so as to be normal to the taper, hence minimising any loss of light due to rays entering the taper at angles outside the maximum acceptable angle $\theta=\sin^{1}(NA)$, where NA is the numerical aperture of the taper, (equal to the magnification), ie 24.6/70.7 = 0.348 in the example given above.

In a preferred arrangement, efficient light gathering is achieved by bringing the sample as close as possible to the imaging lens and arranging the lens powers to cause the cone of light entering the fibre optic taper just to fill the numerical aperture (NA) of the taper.

In a preferred embodiment, the taper is 110mm diameter with a demagnification of 2.87. F1.1 lenses are preferred.

Preferably the imaging lens is a complex lens consisting of a number of separate lens components.

Preferably the light source is a laser light source.

In a further arrangement a second field lens may be mounted close to the sample plate to select rays generally normal to

the plate, even near to the edge of the plate, and thereby minimise parallax effects.

Each or both of the lenses may be a simple single element lens or to advantage may be a multi-element lens.

Comparison of different arrangements

An example of one arrangement using a bare cooled CCD is shown schematically in Figure 1(a). A typical 1" CCD will have 1024x1024 pixels, the pixels being of size 24 microns x 24 microns. A shutter (or iris) is shown included in the light path to protect the CCD if a strong light source eg a laser, is being used to excite fluorescence in the sample, particularly if a time-resolved fluorescence method is being used (ie light on, light off, read sample, repeat).

In other circumstances, the shutter or iris may be employed to reduce frame shift smear such as when using self luminescent samples.

An alternative to lens imaging is contact imaging in which a sample is presented directly to the CCD, via either a one-to-one thin fibre-optic plate to which the CCD may be bonded, or via a demagnifying taper to which the CCD may be bonded. This is shown in Figure 1(b), in which a taper (typically having an input diameter of 70mm) enables a plate to be viewed in four shots (or exposures). Contact imaging is to be preferred where higher light capture efficiency is desired. Figure 1(b) shows a bare cooled CCD but an image intensified CCD could instead be used.

The relative light gathering efficiency of lens imaging and taper contact imaging is shown in Figures 1(a) and 1(b). In the case of a lens, the standard formula (in terms of the demagnification m and the ratio F of focal length:diameter) given in Figure 1(a) applies. This means that in the example

given an overall efficiency of only about 0.7% is obtained even with a very high quality F1.1 lens. The lens has the advantage that it can view the sample in one go. Also a shutter can be included as mentioned above. With taper contact imaging as depicted in Figure 1(b), an efficiency of about 3% is obtainable, ie 4-5 times higher than for the lens. In the taper formula a factor 0.7 arises from the packing fraction of the fibres in the taper, a factor 1/4 comes from the fact that 4 shots are needed to view the whole plate, and there is a factor $1/m^2$, where m is the taper demagnification.

A preferred embodiment of lens-taper imaging embodying the invention is shown in Figure 2 in which a field lens bends the rays so as to be normal to the taper so as to reduce light loss. The imaging lens focuses the well plate onto the CCD camera. The lens powers are selected so that the cone of light entering the taper just occupies the numerical aperture of the taper. A shutter is employed for the reasons given above.

Figure 1(c) contains a formula giving the efficiency of the arrangement shown in Figure 2 for m=4.47, and demonstrates that an efficiency of 3.5% can be obtained which is greater than either lens or taper imaging when used alone.

It is to be noted that another field lens of appropriate strength also could be inserted advantageously in front of the sample plate, that is between the sample plate and the imaging lens. This additional field lens would receive light rays on average normal to the sample, and direct them to the imaging lens. This reduces parallax effects which can result in the loss of light being received from deep in the wells of a sample plate.

Figure 3 shows a second field lens mounted just beneath the sample plate which collects rays that are on average normal to the plate. This collection of normal rays occurs across the whole area of the plate, even at the edges. As described above

the arrangement minimises parallax effects, which can make it difficult otherwise to gather light from deep down a sample plate well. The imaging techniques illustrated here may be used to advantage in systems for analysing photon emitting assays such as described in UK Patent Specification No. 2294319.

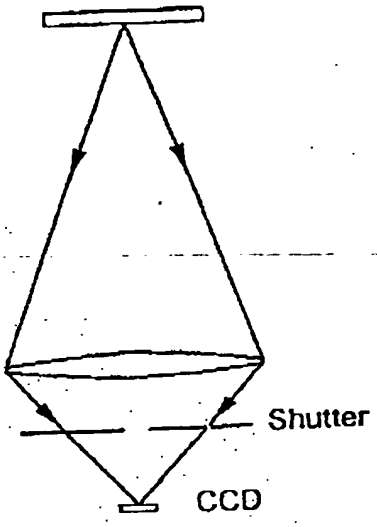
Fig 1

Imaging Whole Plate (schematic)

(m= 110/24.6 = 4.47 for 1 shot)

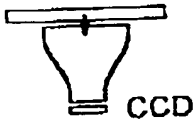
(a)
Lens

(1 Shot)



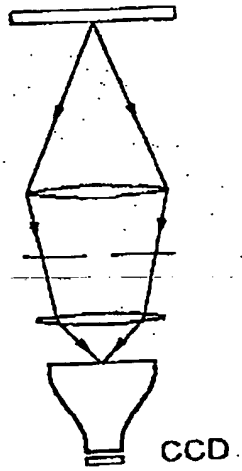
(b)
Taper Contact

(4 Shots)



(c)
Lens-Taper

(1 Shot)



Efficiency
(for plate)

$\frac{1}{4F^2(1+m)^2}$

for m=4.47 and F=1.1

= 0.69%

$\frac{0.7}{4m^2}$

for m=58.4/24.6 = 2.373

= 3.1%

$\frac{0.7}{m^2}$

for m=4.47

= 3.5%

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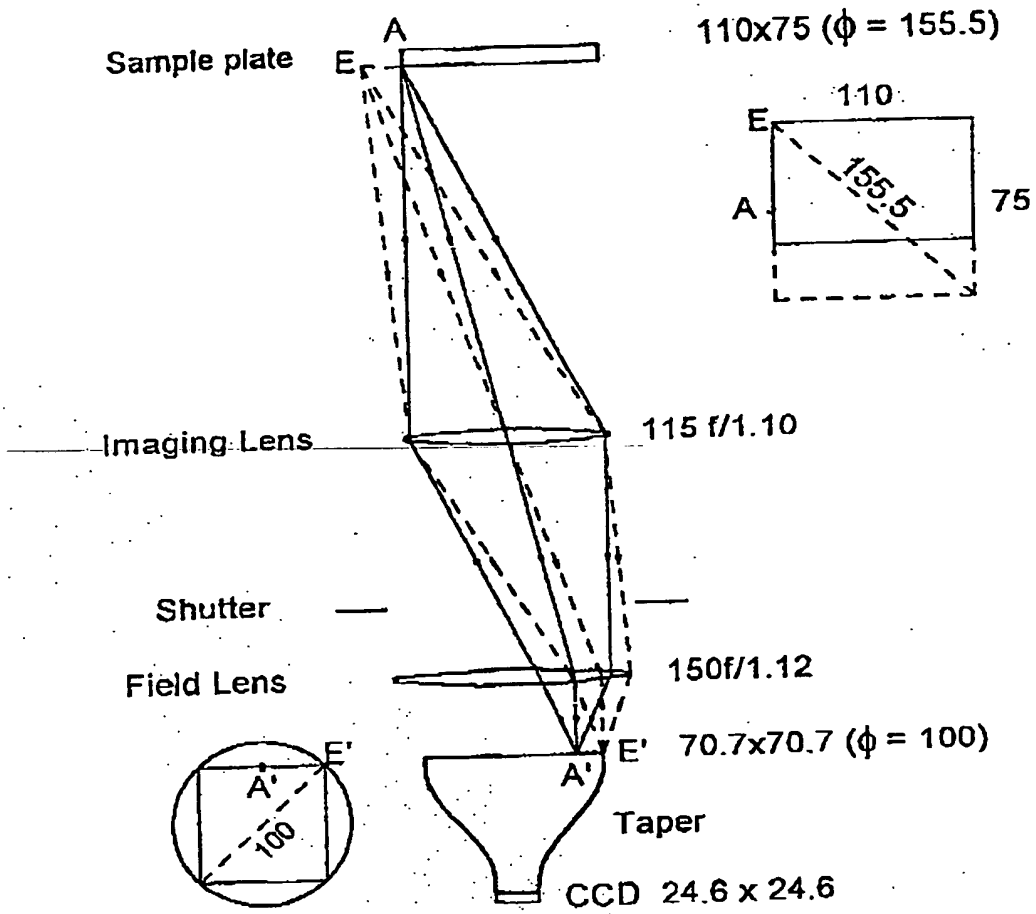
Fig 2

Lens - Taper

Viewing whole plate (Schematic)



Distance - Plate to Taper = 434mm

Field of view = 36.6 degrees.

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Fig 3

Lens - Taper Viewing whole plate (Schematic)